

(2) The histological picture and the sequence of pathological developments do not show any significant differences from the appearances seen in epidemic hepatitis or the hepatitis after serum injections.

(3) The histological appearances do not support the suggestion that either syphilitic lesions of the liver or arsenobenzol poisoning play any part. The appearances are more compatible with damage by an agent similar to that causing serum jaundice or epidemic hepatitis.

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## THE USES AND LIMITATIONS OF THE SERUM TESTS FOR SYPHILIS\*

BY

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We have ventured to change the title of this paper from that originally selected to the present one because the great increase in the practice of serum-testing for syphilis as a matter of routine in medical examination, which has occurred in the last few years, has made it important that someone in this country should stress the limitations of these tests and emphasize the fact, almost a commonplace with serologists, that a positive reaction with such a test is not a verdict on the question of syphilis in the donor of the blood specimen.

We propose to discuss the serum tests for syphilis under the headings of their uses and limitations in diagnosis and of their use as a guide to the management of syphilis. In doing so we do not intend to present anything approaching a complete review of the literature, but merely to present evidence, either in our own or in other workers' experience, sufficient to prove the points which seem important.

First as to the use of these tests in diagnosis.

#### The use and limitations of serum tests for diagnosis of syphilis

We think it would be profitable to discuss particularly the causes, prevention

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and detection of false positive reactions and the prevention of a false diagnosis of syphilis when a positive serum reaction is not supported by the clinical evidence.

In 1918, the Medical Research Committee's (now the Medical Research Council) Committee on the Standardization of Pathological Methods said :

*“ In the opinion of the Committee, there is no process of bio-chemical diagnosis that gives more trustworthy information or is liable to a smaller margin of error than the Wassermann test when it is performed with completeness and with proper skill and care.”*

This is probably as true today as it was when written a quarter of a century ago, but it is equally true that no group of tests has given rise, through unskilful performance and through inadequate appreciation of their limitations, to more unhappiness than have the serum tests for syphilis. The increase in the practice of including such tests in general medical examinations, for example, of pregnant women, candidates for marriage, blood donors, students for matriculation, applicants for work, and so forth, and the increased sensitivity of the methods employed by some workers have lengthened remarkably the list of non-syphilitic conditions liable to cause false positive reactions, either with tests inefficiently performed or, though admittedly to a smaller degree, with the best tests we have. In fact the results have shown that the interpretation of reports on serum tests for syphilis is by no means so free from pitfalls as it appeared to be only a few years ago, and it is probable that routine blood-testing has led to large numbers of persons being subjected to anti-syphilitic treatment under diagnoses of syphilis which further investigation would have proved to be false.

A non-specific reaction may be due to a fault in the method, to an error in carrying out the technique of the method, to a peculiarity of the patient, or to two or all of these factors. The above quoted Standardization Committee qualified its eulogy of the Wassermann test with the stipulation, “ when performed with completeness and with proper skill and care,” and it is not difficult to justify the stipulation. As reliability of technique is of paramount importance, we wish to devote some space to the subject, particularly to show present-day defects and the need for continual effort to raise and then maintain the standards of methods and of technique.

Of the *Wassermann test* it was said long ago that there were almost as many methods as laboratories in which they were practised, and every worker considered his own method to be as good as any and better than most. Clearly it was most desirable to discover which was the best, that is the most sensitive and yet specific ; with this object in view, one of us, at the League of Red Cross Societies' Northern European Conference in 1921, suggested a comparison of the different test methods employed in the principal laboratories of the world. The idea was taken up and elaborated by the Health Organization of the League of Nations, first by arranging for a few flocculation tests to be compared with one another and with the routine Wassermann employed in a number of laboratories in Austria, Belgium, Denmark, France, Germany, Poland and this country, and later by means of the laboratory conferences in Copenhagen in 1923 and 1928, and in Montevideo in 1930. The first comparison, in which each laboratory tested the different methods on locally supplied sera, was a failure as a means of evaluating the merits of any published method, but it did show how much individual workers of the highest repute can and do modify the technical details of a flocculation test, however carefully its author may have described them.

The comparisons carried out at the two serum conferences in Copenhagen and that in Montevideo killed a number of methods which had been believed by their authors to be better than any other and demonstrated quite clearly that very few pathologists can evaluate their own methods. It was quite astonishing to see there how some pathologists of good standing seemed to have lived in a veritable fool's paradise in regard to the reliability of their methods. Presumably when the results had not accorded with the clinical findings—that is, if they were not working in laboratories far removed from clinics and quite out of touch

with the clinical side—they had persuaded themselves that the clinicians must have been wrong. The experience demonstrated quite clearly that a method and a technique can be judged most efficiently by the acid test of a comparison with a number of other methods on unknown sera from cases that have been diagnosed by well-experienced clinicians, the results being judged, moreover, by impartial, unprejudiced observers.

This truth was early put into practice by one of us in the comparisons, carried out from 1924 until the outbreak of the present war, between a standard method employed in a laboratory set up by the Ministry of Health for the purpose and the methods employed in the different laboratories approved for testing specimens under the Public Health (Venereal Diseases) Regulations 1916; the same method of evaluation has in more recent years been employed on a much larger scale in the U.S.A., with remarkable success as judged by the improvements in the standards of testing in the participating State laboratories.

We should like to mention very shortly some details of the comparisons carried out in this country and in the U.S.A. to illustrate some points of importance.

Those in this country apply only to the Wassermann test, and the yardstick by which the methods employed in the different approved laboratories were judged was what was originally known as No. 1 Method of the Medical Research Committee's Special Report Series No. 14 (1918); later it was described in careful detail by the late Dr. E. J. Wyler (1929) in Medical Research Council's Special Report Series No. 129, and is now commonly known as the "Harrison-Wyler Method." In the comparisons at the Copenhagen Conferences in 1923 and 1928 this method proved the most sensitive of those depending on complement fixation which did not give any false positive reaction, and in the comparison in Montevideo it proved practically equal to the most sensitive yet specific of the complement fixation methods; here it should be mentioned that, at this conference, all methods gave a certain percentage of positive reactions with sera from lepers. Except for these reactions, at the three conferences it gave no positive reaction with 1,057 non-syphilitic sera.

The comparisons in this country were instituted by the Ministry of Health because whenever it was suggested to the serologist in an approved laboratory that his method would probably be too insensitive or too sensitive, according to the case, he could ask for proof. After 1924 the Ministry of Health never argued on descriptions of methods, but invited serologists in turn to collaborate in comparisons of their methods with the Harrison-Wyler as practised in the laboratory of the Ministry of Health first by Dr. Wyler and later by Dr. G. M. Richardson. The comparisons were arranged as follows. Specimens of blood were supplied by the St. Thomas's Hospital Venereal Diseases Treatment Centre to the Ministry's laboratory, under identification numbers other than those of their donors in the clinic and unaccompanied by any clinical data. In the Ministry's laboratory the sera were separated, and portions of each specimen sent to the laboratories collaborating in the comparison, usually in batches of twelve; as far as possible also, an endeavour was made to arrange for the tests to be carried out on the same days. At first the number of specimens tested in a comparison was only a hundred; later it was increased to two hundred. When this number of specimens had been reported upon by the Ministry's laboratory and any laboratory collaborating in a comparison of tests on the same specimens, the results of the tests in both laboratories, together with the clinical data, were sent to the two laboratories, with a summary and possibly a commentary.

According to the records that are still available—most have been sacrificed on the altar of salvage—in 3,883 tests of sera from persons judged not to be suffering from syphilis the Harrison-Wyler method as practised in the Ministry's laboratory gave two positive reactions. One of these was with serum from a patient with a penile sore, and the donor's serum was positive in other laboratories on repeated tests, but the reaction became progressively weaker without the intervention of any treatment, and the final verdict was against a diagnosis of syphilis. In the other case the blood gave either positive or doubtful reactions with

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repeated tests in other laboratories and may have been of the type to be discussed more fully below. Thus on the record of tests in the three League of Nations conferences and in the numerous home ones this method would appear to have been almost 100 per cent specific except with sera from lepers. Yet even in the best hands it has not proved perfectly specific; with specimens which did not happen to have been included in those used for these comparisons it has given occasional false positives, as we shall show later.

Here it is necessary to say that, in the evaluation of a test method from the point of view of specificity, we do not agree with the penalizing of doubtful reactions in non-syphilitic cases because, in respect of interpretation of results of serum tests for syphilis, our practice has been in accordance with the following recommendations adopted by the Health Organization of the League of Nations (1932) at its nineteenth session.

*“All reactions which are neither unquestionably negative or unquestionably positive should be reported as  $\pm$  or ‘doubtful.’” And, “The sign  $\pm$  means that the tests performed do not enable any definite pronouncement to be made as to whether the reaction is to be termed ‘positive’ or ‘negative.’ If the patient is known to have been infected with syphilis, the reaction may be regarded as positive. If there is no history or clinical evidence of syphilis, the serological result indicates the necessity of making a particularly careful clinical examination of the patient and of sending in a repeat sample. If the result is once again  $\pm$ , the reaction should be regarded as having no diagnostic value.”*

As regards sensitiveness with syphilitic sera, in 107 comparisons with the methods employed in individual other laboratories in this country, the Harrison-Wyler method proved more sensitive than that of the other laboratory 88 times. Of the 19 laboratories whose methods appeared more sensitive than the Harrison-Wyler, 10 reported false positives (36 in all with 912 non-syphilitic sera). The 9 laboratories which reported more positive reactions with syphilitic sera but none with non-syphilitic reported with 933 syphilitic sera, 328 positive and 102 doubtful reactions (that is 430 positive and doubtful reactions, which could all be regarded as positive in accordance with the League of Nations recommendation quoted above), as compared with 248 positive and 192 doubtful (that is 440 positive and doubtful reactions) by the Harrison-Wyler method, so there was very little in it. Incidentally with reference to the figures just quoted, it is not valid to compare them with any obtained by other methods in other countries because there can be a world of difference in reactivity between any two batches of syphilitic sera, and no complement fixation method employed in this country has ever been compared on the same sera with any employed in the U.S.A. It is true that the Wassermann used here has been compared with the Kahn performed by its author, and in turn the Kahn has been compared with Wassermann methods practised in the U.S.A., but the results do not form a basis for comparison of the complement-fixation methods employed in the U.S.A. with ours, because comparisons such as these are affected very greatly by the amount of treatment the donors of the syphilitic sera had received and by the sources of the control sera. But this is only incidental and not relevant to our present discussion.

As an indication of the variations in technique which workers tend to introduce into the practice of a given, well-described method we may mention that although many workers in this country say they use the Harrison-Wyler method of the Wassermann, very few of them obtain results as sensitive and as specific as those in the Ministry's laboratory; in fact, it appears that, with only one or two exceptions, comparable results have been reported only by workers who learnt the method at its birthplace or in the laboratory of someone who did. The method was described with particular care by the late Dr. E. J. Wyler in the Medical Research Council's Special Report No. 129, with the object of enabling other workers to copy it exactly, but the experience shows more clearly than anything that a method can hardly ever be learnt properly from a book description. It justifies the action of one of us at the outset of all these comparisons, in arranging that Dr. Wyler should go to the laboratories of the authors of the Meinicke,

the Sachs-Georgi and the Sigma tests respectively, which were the tests then under comparison, and practise each in its birthplace until he was obtaining results according exactly with those of its author.

The wide variation of results which can be obtained with what purports to be the same method applied by different workers to the same sera, is shown well also in some comparisons organized in the U.S.A. by the Committee on Evaluation of Serodiagnostic Tests for Syphilis. In a comparison arranged in 1935 (Parran et al. 1937) the "Kolmer-Wassermann" in 15 different hands gave percentages of positive reactions with the same syphilitic sera varying from 35 to 71, the percentage obtained by Dr. Kolmer with the same sera being 59. With non-syphilitic sera the same workers obtained percentages of positive reactions ranging from 0 to 3.0. In the same comparison the "Kahn Standard Test" was applied to the same syphilitic sera by 13 different laboratories, with positive results ranging from 37 to 83 per cent, Dr. Kahn obtaining 75.7 per cent; with non-syphilitic sera the positive reactions ranged from 0 to 2 per cent. As showing the improvement which can be effected through the demonstration of deficiencies by such comparisons, in one organized in 1942 by the same Committee (Parran et al. 1942) the percentages of positive reactions obtained by 16 laboratories with the "Kolmer-Wassermann" test applied to the same syphilitic sera varied from 68.8 to 87.0, Dr. Kolmer's laboratory obtaining 84.9 per cent. In the same comparison the percentages of positive reactions with the "Kahn Standard Test" applied to the same syphilitic sera ranged from 54.7 to 86.1, Dr. Kahn's laboratory reporting 77.7 per cent. We should like to take this opportunity of congratulating the American Committee on the great improvements in technique resulting from their comparisons, which, if we may say so, seem to have been triumphs of organization.

Variations in results of tests by what purport to have been the same methods depend on a number of factors, including confusion of specimens, variations in and unreliability of reagents, modifications of technique, and errors in technique.

Confusion of specimens is a matter of laboratory organization; it requires no further comment than to say that any system of preparation for testing, or for actual testing and reading of results, which is attended by any danger of specimens being confused once in several thousand cases should be reformed.

As regards reagents, it goes without saying that not only should the chemicals of fixed composition be of the purest but should not be exposed to contamination by the use of dirty glassware. Organic reagents are perhaps a more difficult matter. We believe that as far as possible such reagents as extract, haemolytic amboceptor (we strongly prefer rabbit versus sheep), and complement serum should be obtainable from a central source, and in this connexion, the standardization of complement serum which has become possible through the use of Richardson's (1941) simple method of preserving it in the liquid state is most welcome. An enquiry which one of us carried out early in the war to discover how the approved laboratories were likely to fare in respect of the supply of guinea pigs raised a very strong suspicion that a number of them had been using complement serum of poor quality. Since then Dr. Richardson has been able to supply laboratories with serum of suitable titre and free from natural haemolytic amboceptor. This complement remains good even under quite adverse climatic conditions.

With regard to the question of technique, we believe that every pathologist should learn first *in the laboratory of its author*, a method which has stood up well to the acid test of such a comparison as we have mentioned, that he should practise it with the closest watch on its agreement or otherwise with the clinical evidence—resisting here any tendency to wishful thinking—and that he should introduce modifications only after these have been tried out in parallel on some hundreds of specimens. When the itch to modify troubles a worker, he should remember the following extract from the "Recommendations regarding Serological Syphilis Tests" adopted by the Health Committee of the League of Nations at its nineteenth session (1932):

*"Owing to the increasing number of different methods and their more or less*

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*important modifications, it is almost impossible to judge their comparative values from the reports published concerning them. Even when a number of methods are tested by well-trained pathologists in different laboratories, a false idea of their values may be obtained owing to the modifications, often apparently trivial (our roman) which the pathologists introduce into the technique laid down by the authors of the tests. This was clearly shown in the course of the simultaneous trials carried out in a number of well-known laboratories at the instigation of the Health Organization of the League, when the testing of a limited number of methods produced widely divergent results. Whereas, in one laboratory a certain method yielded the best results, in a second laboratory, another was found more satisfactory; in no case did the results coincide, even when the tests were performed with samples received from a central laboratory."*

All experience justifies the recommendation of the Standardization Committee of the Medical Research Committee in 1918, that the laboratories in which these tests are carried out should be few rather than many. We would add that those few should be in close touch with large, first-class treatment centres. In any case the reliability of every method should be checked periodically by submitting it to comparison with some standard, on the lines already described. So much for technique. Assuming, however, that the technique is satisfactory, can we say that a positive reaction, repeated on two or more specimens, using two or more of our most specific tests (say, speaking for workers in this country, the Kahn Standard Test and the Harrison-Wyler and the Richardson complement fixation methods) means that the patient is suffering from syphilis, provided that the following can be excluded: yaws, leprosy, trypanosomiasis, relapsing fever, malaria, scarlet fever, tropical ulcer, pellagra, beri-beri, pneumonia, late tuberculosis, diabetes mellitus, enteric fever, sclerodermia, or malignant tumour, to enumerate some of those mentioned in text-books as having in various hands given positive reactions. More recent work has shown that we must add more to the list and perhaps the most important of these are glandular fever and recent vaccination. In the case of glandular fever, Kaufman (1941) found that the reaction could persist for as long as two months. The effect of vaccinia in provoking a positive serum reaction was apparently first suggested by Moore in 1941, and has been confirmed by Lynch, Boynton and Kimball (1941) who obtained 29 positive and 14 doubtful reactions with the blood of 263 recently vaccinated youths who prior to the vaccination were negative. Also Thomas and Garrity (1941) found 26 positive reactions in 10,000 naval recruits tested 12 days after vaccination, but only 6 in another 10,000 tested before vaccination. The authors of these papers think that if the test had been carried out on each of the subjects more often, a higher percentage of positive reactions would have been obtained.

Mohr, Moore and Eagle (1941) have reported in detail on 11 cases out of 200 seen in the past 15 years in which, without evidence of syphilis, the blood has given positive syphilitic serum reactions. The 11 included subacute inflammation of unknown origin, acute labyrinthitis, vaccinia, pneumonia (four cases), sore throat of unknown origin (two cases), glandular fever and rat-bite fever.

But perhaps more misleading than the conditions already mentioned is a transient broncho-pneumonia which apparently gives rise to very little constitutional disturbance and may cause a positive reaction persisting for two months or longer. Particular attention was drawn to this condition by Fanconi (1936) who reported positive reactions in three under-nourished school children and one convalescent from measles who had suffered, or were suffering, from what was described as "hilifugal broncho-pneumonia" in which radiograms showed a streaky infiltration of the lungs. The serum reactions persisted until the radiographic appearances had become normal, that is for a number of weeks. Fanconi quoted Forssman (1932) as having found only five false positives in 7,711 general clinic cases, but three in 633 cases of pneumonia, and Pockels (1933) who had found four false positives in 206 convalescent measles cases. Fanconi's observations were later confirmed by Hegglin and Grumbach (1941) and by Jahnel (1941).

Hegglin and Grumbach had 18 cases of a typical, almost afebrile, pneumonia with the streaky infiltration mentioned by Fanconi and positive syphilitic serum reactions, and Jahnel reported on 11 similar cases. Both his and Hegglin and Grumbach's cases had a high proportion of Pfeiffer's influenza bacilli in the sputum, and their sera strongly agglutinated this organism. It seems possible that Pfeiffer's bacilli may be capable of provoking pseudo-syphilitic reactions in patients with labile serum.

Chargin and Rein (1941) subjected to the Kahn Verification test (1940), to which we shall refer in more detail later, a large number of sera from patients suffering from a considerable variety of ailments, and their tables show a rather strong tendency of various dermatoses, infectious fevers, Vincent's angina and tuberculosis to make the blood more reactive with extracts used in syphilitic serum tests.

As may be expected, some tests are more liable to give false positives in these conditions than are others. Thus in a comparison of serum tests carried out in the U.S.A. in 1935 (Cummins et al.) the percentages of false positive reactions obtained by 13 pathologists each employing his own method varied from 42 to 72 in leprosy; 0 to 7.7 in tuberculosis; 0 to 9.7 in malignant disease; 0 to 8.9 in fever other than malaria, natural or induced; 8.6 to 20.6 in malaria; 0 to 3.9 in jaundice; and 0 to 3.8 in pregnancy.

Even the long list of conditions which we have given does not exhaust the pitfalls. Chargin and Rein to whose paper we have referred mentioned in it tests of 253 specimens from what they called "problem cases," in which the blood had sometimes been positive and sometimes negative and nobody had been able to say whether or not the persons concerned had ever had syphilis. Also Eagle (1941) has mentioned that in 40,545 tests of students he found a non-specific reaction not due to technical error or accounted for apparently by any of the diseases mentioned above once in 4,000 times.

Our own experience of such apparently abnormal cases is limited, but we hope that Dr. Richardson, in the discussion following this paper, will supplement it with some observations on tests of sera supplied by one of us and by other workers. One we will relate here as it exemplifies well the danger of making a false diagnosis of syphilis when none of the pathological conditions already mentioned can be cited as accountable for positive serum reactions.

A virgin aged 50, with no history suggestive of either congenital or acquired syphilis, volunteered as a blood donor, but her serum was found to give positive reactions to syphilitic serum tests. The tests were repeated on fresh specimens and found to be positive in two other laboratories. Accordingly the patient was referred for advice to one of us, who took a fresh specimen parts of which were sent to Dr. Orpwood Price and to Dr. Richardson. Dr. Price obtained negative reactions by the Harrison-Wyler method of the Wassermann, and by the Kahn test not only with that specimen, but with another sent to him six weeks later from the same patient. Dr. Richardson's results were very interesting; by the Harrison-Wyler method and by the Richardson modification of that method, using *human heart extract*, the results were positive, but with *ox-heart extract* the Richardson modification (1940), which weakens non-specific reactions and strengthens specific ones, gave an almost negative reaction, the Harrison-Wyler method with the same extract giving a single plus reaction. The Kahn test gave a doubtful reaction at room temperature and at 0° C. but a completely negative one at 37° C. The suggestion therefore was that the positive reactions which had been obtained were non-specific, and no treatment was advised. Exactly similar reactions were obtained six weeks later, and a month after that the Harrison-Wyler method gave with five different human heart extracts reactions ranging from single plus to double plus, whilst the Richardson modification with the same extracts gave reactions ranging from doubtful to double plus. With four different ox-heart extracts the Harrison-Wyler method gave with the same specimen reactions ranging from doubtful to single plus, while those with the Richardson

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modification ranged from negative to doubtful. The Kahn reaction was again negative at 37° C.

The explanation of the difference between Dr. Price's and Dr. Richardson's results with the Harrison-Wyler method in this case appears to lie in the human heart extracts as Dr. Price uses perfectly fresh heart and Dr. Richardson, according to the prescription of Dr. Wyler, uses heart which has hung for about 36 hours.

We understand that Dr. Richardson has had some similar specimens from blood donors, and it seems clear that we must recognize the existence, in some apparently normal people, of a certain lability of the blood serum which may cause it to give positive reactions in syphilitic serum tests (either permanently or under the influence of intercurrent disease) and more with some tests than with others, although the former may differ only slightly from those which in the same circumstances have proved faultless.

Such a lability has long been recognized as a natural phenomenon occurring in a certain proportion of rabbit sera, and there seems to be no reason why it should not occur, though to a smaller degree, in human.

In connexion with the lability of rabbit sera in this respect, some recent observations by Albrecht (1942) are interesting. He found that of the normal rabbits at his disposal, 1.21 per cent gave a definite reaction with the Chediak dried-blood modification of the Meinicke test, and that, so far as the provocation of reactions in other rabbits or increase or weakening of the reactions in those already giving positive ones were concerned, the condition was uninfluenced by heat or cold, over-feeding or starvation, or by any intercurrent affection such as catarrh or gastritis.

The question arises : how are we to guard against a false diagnosis of syphilis in a case with no sign or history suggestive of syphilis ?

First as to routine tests, as Sachs (1942) recently pointed out, the theoretical ideal of sensitivity capable of detecting the least trace of syphilitic reagin with complete specificity is unattainable by any present known method because of the presence in all tissue extracts of the "ballast material" with which the very minute amount of specific antigen is associated and which may in certain circumstances react with a serum under test in the same way as does the specific antigenic fraction with syphilitic reagin ; such a condition is particularly apt to occur in conditions which increase the blood sedimentation rate.

The serum test for syphilis must be a compromise, and for the standardization of the reagents to avoid non-specific reactions it is clear that the control non-syphilitic sera should emanate from persons suffering from pathological conditions rather than from normal persons. Thus Kahn (1941) in a criticism of recent comparisons pointed out that owing to the use of normal sera as controls, the Presumptive Kahn test had appeared superior to the Kahn Standard test because, whereas it had given a higher percentage of positive reactions with syphilitic sera, it had given no false positive. Experience in the routine work of a hospital's clinical laboratory had, however, shown the Presumptive test to be insufficiently specific for the positive diagnosis of syphilis. As mentioned above, the method employed should have stood up well to the acid test of a comparison on unknown sera.

With standards based on non-syphilitic pathological sera as controls, can more be done ? With regard to the Harrison-Wyler modification of the Wassermann test, undoubtedly every serum which gives a positive reaction conflicting with the clinical evidence should be subjected to the Richardson modification, which, as said, weakens non-specific reactions and strengthens specific ones. Here we may say in support of the value of the Richardson modification that for some months before the outbreak of the present war one of us was collaborating with the Health Organization of the League of Nations in preparing for another serum conference to be held in September/October, 1939, and the preparation included the collection of a number of problem sera to be tested by the participants in that conference. The sera were being tested in the Copenhagen Institute, in Paris



by Debains, and in London by Richardson, and just before the war began a letter arrived from Copenhagen conveying congratulations on the results of the Richardson method.

Further, it is clear that every serum which gives an unexpected reaction should be subjected to the Kahn Verification test, which depends on the fact that a non-specific reaction is weakened at 37° C., as was pointed out by Sachs long ago, and is strengthened at 0° C.; conversely specific reactions are strengthened at 37° C. and weakened at 0° C. Whether or not the Kahn verification test will always provide safety is uncertain. Chargin and Rein's 253 problem sera which we mentioned above were from about 20,000 patients who had been examined by the authors. The Kahn Standard test gave with them 36 negative reactions, 101 doubtful, and 116 positive. The Kahn Verification test carried out in Kahn's laboratory gave with the same specimens, 83 syphilitic reactions (62 had been positive and 20 doubtful by the Standard test), 85 of the general biologic type (37 of these had been positive with the Standard test), 63 negative (three had been positive and 34 doubtful to the Standard test); and 22 inconclusive. The authors were still not able to say if the 83 that gave syphilitic reactions with the Verification test were in fact from syphilitic persons.

It seems clear from the above and from our own experience that the Kahn Verification test will help to decide the non-specificity of some of the reactions given by these problem cases, though we are still not in a position to say that a positive reaction given by it certainly means that the patient is syphilitic.

What then should be the procedure in the case of a positive reaction to a diagnostic test which is not supported by any clinical data? We suggest the following:

(1) Exclusion of laboratory error by submission of another specimen, which should also be tested in at least one other laboratory.

(2) In the event of the positive reaction being repeated, application of a Richardson complement fixation and of a Kahn Verification test.

(3) Careful enquiry respecting any recent illness due to any of the conditions already mentioned as being liable to cause false positive reactions.

(4) Further clinical examination, including radiography of the cardio-vascular system and tests of the cerebro-spinal fluid.

(5) Examination of siblings. If none of these examinations discloses anything definite, it seems possible that circumstances would justify one in withholding treatment whilst further tests were carried out over a period of many weeks.

In any case we hope we have said enough to justify the advice that when the history and clinical evidence are opposed to positive results of serum tests for syphilis, a diagnosis of syphilis should not be made at once. It should be remembered that the patient may be one of those rare persons who have a labile serum (as have many rabbits) and that a consultation may be a wise procedure before it is finally decided to start treatment.

#### **The use of serum tests for guidance in the management of syphilis**

In the use of serum tests as an indication of the effect of treatment, we would draw attention to some points of importance, viz:

- (1) The variability of the duration of positive reactions after apparent cure, and
- (2) The superiority of quantitative tests in showing the effect of treatment.

As regards the duration of positive reactions after cure, it has recently been shown in early cases apparently cured by such intensive methods as the multiple injection that there is a wide difference between the periods during which the blood has remained positive after completion of the treatment. For example Rose, Simpson and Kendell (1942) in 23 cases apparently cured by a single session of pyrexia in conjunction with multiple injections of mapharsen during one day, found that the period of subsequent positivity ranged from 21 to 176 days. It follows from this that in comparing one treatment with another, one should not take into account results of serum tests performed less than six months from

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the start, since even if a patient with early syphilis has been cured by a single day's treatment his blood may remain positive for as long as six months. Although Rose, Simpson and Kendell found in their early cases that the duration of positivity depended directly on the strength of the serum reactions at the start of treatment, it still seems possible that in old-standing cases the persistence of positivity might be a matter of tissue habit, as was first suggested by one of us at the International Medical Congress in 1913. Otherwise it is difficult to explain those cases in which treatment has been stopped when the blood was still positive but in which tests years later have shown that by that time the reactions have been reversed to negative.

In the management of old-standing sero-positive cases we have both for many years pointed out the advantages of quantitative titration of the strength of the reaction. If a mere qualitative test is applied, no effect of the treatment is apparent for course after course, and all concerned are correspondingly disheartened. If, on the other hand, such a quantitative test as the Sigma of Dreyer and Ward (1921) is applied, the effect is often seen at the end of the first course, and a steady diminution in the strength of the reaction from course to course is decidedly encouraging. More recently Moore and Eagle (1941) and also Simpson, Rose and Kendell (1942) have drawn attention to the same fact. If a quantitative complement fixation test is employed, probably it is best to make the successive dilutions of the patient's serum with a pooled normal serum. Of the flocculation tests, probably the Sigma provides the finest gradation, but the Sigma is not so sensitive as the Kahn, and it may be that the Kahn quantitative method (1925) in which the strength is determined by multiplying by four the dilution of the serum which causes standard flocculation may give sufficient information. The method of Wadsworth, Maltaner and Maltaner (1938) appears rather complicated for general use and that of Vernes (1920) requires too much expensive apparatus.

### Summary

In the space at our disposal we have not, of course, been able to cover the whole ground of the uses and the limitations of the serum tests for syphilis, but we hope we have established the importance:

(a) Of raising and maintaining the general standard of serum testing to the desirable compromise of maximum sensitivity combined with maximum specificity, through the use of carefully standardized reagents, thorough adequate training of testers and through comparisons on unknown sera;

(b) of a proper appreciation of the limitations of these tests and a clear idea of the steps to be taken when the positive result of a test is not in accord with the clinical evidence; and

(c) of the value of the quantitative tests in the control of treatment.

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## DISCUSSION

Dr. David Nabarro said that he felt a good deal less certain about the reaction now than he did thirty or more years ago when he started doing these tests; as knowledge increased, footholds seemed to become less secure than they were at first.

Many points had struck him during the years in which he had been doing this work; one of the most important was the actual carrying out of the tests. Col. Harrison mentioned that the technique should be above reproach, but was that so in actual practice? If Col. Harrison visited some of the laboratories in this country he would be shocked by the way in which some of these tests were carried out. There were individual pathologists who introduced modifications into the technique which he thought should not be allowed. Serological reactions should be carried out under the strict supervision of the Ministry of Health, such laboratories being licensed for the purpose and staffed by people who had had special training and were checked from time to time in the way in which Col. Harrison had indicated. He himself had had some experience of Col. Harrison's supervision in days gone by. These checks should be done periodically to eliminate slackness.

With regard to one or two of the cases which had been mentioned, Dr. Nabarro saw a blood donor last year, a young girl of eighteen who looked the picture of health, and who gave a strongly positive Wassermann reaction. The test was repeated, and the same result was obtained. She was severely rated by her parents for having misbehaved herself, this she denied, and the history was gone into very thoroughly, when it was found that she was congenitally syphilitic. Her teeth were suggestive of syphilis to him before he went into the history. He thought that some of these cases of young adults born in the last war whose fathers might have had enough treatment to allow the child to be born apparently non-syphilitic would be found to be recognizable congenital syphilitics.

Cases of dilated aorta and aortitis were sometimes seen in which the Wassermann was negative, yet the individuals had had syphilis, so that there were two fallacies to think about—the "false" positive and the "false" negative. Even if the Wassermann and Kahn test were negative in a case of syphilitic aortitis, the patient must be regarded as syphilitic, and in the event of a fatal issue, syphilis as a cause of death should be stated on the death certificate in order that the Registrar-General's figures might be the more accurate from the point of view of venereal diseases.

Ravaut wrote many years ago that a Wassermann reaction had only the value of the signature attached to it; that was very true, as true then as it is today. The clinician must know who had done the Wassermann test before he could decide its value.